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PRINCIPAL INVESTIGATOR: Daniel D. Carson, Ph.D.

CONTRACTING ORGANIZATION: University of Delaware
Newark, Delaware 19716

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6. AUTHOR(S)
Daniel D. Carson, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Delaware
Newark, Delaware 19716

E-Mail:

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13. ABSTRACT *(Maximum 200 Words)*

Mucin glycoproteins are highly expressed by many tumors, reduce normal cell-cell and cell-extracellular matrix adhesion and protect cancer cells from attack by the immune system. Mucin expression not only increases, but also changes from a restricted pattern of apical expression to a general distribution over the entire cell surface. In this regard, conversion of prostate epithelium from a highly-organized, growth-controlled phenotype to a highly proliferative, metastatic phenotype is associated with loss of cell polarity. Very few studies been performed on mucin expression by prostate cancer cells. MUC1 is a large molecular weight, type I transmembrane mucin glycoprotein expressed by normal and malignant prostate epithelium. High level cell surface expression, reported immunosupressive activities of its released ectodomain, and antiadhesive properties all contribute to this mucin's ability to protect and promote tumor cell growth and survival. Recent observations using human breast cancer cell lines indicate that MUC1 can associate with the intracellular signal transducing molecules, β-catenin and GRB-2. Recent studies from the PI's lab demonstrate that cytokines, including interferon-γ, markedly stimulate MUC1 gene expression. Primary prostate tumors are often found in the vicinity of cytokine producing cells, and commonly metastasize to bone marrow, a rich source of these same cytokines.

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Introduction

MUC1 is a large, polymorphic mucin expressed on the surfaces of many normal and malignant epithelia (for review see references 1 and 2). Like other mucins, MUC1 is believed to provide protection to mucosal surfaces from both microbial and enzymatic attack as well as lubricate these cell surfaces. Over the last few years, it has become appreciated that MUC1 functions are more diverse. MUC1 is antiadhesive and inhibits cell-cell and cell-extracellular matrix interactions in both normal, e.g., embryo implantation, and pathological, e.g., cancer cells, contexts. In the case of cancer cells, MUC1 is often highly overexpressed and its antiadhesive properties may promote cell detachment from primary tumor sites as well as protection of the tumor cells from cell-mediated lysis. MUC1 expression is strongly regulated by steroid hormones in breast and uterine tissues *in vivo* and by proinflammatory cytokines *in vitro*.

The large externally-disposed portions of MUC1 (ectodomains) are released from normal and tumor cells where they can both absorb antibodies generated to tumor-specific MUC1 glycoforms as well as suppress immune cell function. Moreover, MUC1 has a transmembrane/cytoplasmic tail region that is highly conserved across species suggesting a conserved function. Recent studies indicate that the MUC1 cytoplasmic tail interacts with important signal transducing molecules, e.g., β-catenin and Grb2, and may participate in signal transduction events (3, 4). In spite of the number of studies of MUC1 expression and function in other systems, very little is known about MUC1 in the context of prostate cancer beyond that it is expressed in normal prostate epithelia and primary tumors. The proposed studies examine the impact of cytokines and androgens on MUC1 expression and function in androgen-sensitive and insensitive prostate cancer cell lines as well as normal prostate epithelia both at the level of gene expression and interactions with signal transducing proteins. A MUC1 gene knockdown approach will be used to disrupt MUC1 interactions with intracellular signal transducing molecules to determine the impact this has on prostate cancer cell growth.

Body

Our research accomplishments are detailed below, following the organization of the original proposal. Some studies have been postponed due to the very low levels of MUC1 expression observed in most of the prostate cancer cell lines chosen for study. We have added studies of normal prostate epithelia to determine if regulation of MUC1 expression is fundamentally different between normal and transformed cells in this tissue.

Task I – To examine interferon-γ and androgen modulation of MUC1 gene expression

We have used both Western and Northern blotting approaches to examine MUC1 expression in LnCaP, C4-2B, and PC-3 cell lines cultured with and without the presence of interferon-γ, TNF-α, dihydrotestosterone (DHT). Only PC-3 cells displayed MUC1 signal and this signal was not influenced by cytokine or hormone treatment. In light of our preliminary observations that MUC1 was readily detectable by immunohistochemistry in normal prostate epithelia and primary tumors, we considered

that loss of expression in the cell lines might be due to: 1) the *in vitro* culture conditions; 2) loss of MUC1 expression in metastatic cells (all three cell lines were derived from metastases) or; 3) the requirement for combinations of cytokines and/or hormones for high level MUC1 expression in prostate cancer cells. We have previously found this to be the case in other cell types. Therefore, we obtained primary cultures of human prostate epithelia (Clonetics) and examined MUC1 expression by Western blotting. These studies demonstrated that combined interferon-γ and TNF-α treatment greatly stimulates both cell-associated and particularly shed MUC1 expression. Thus, normal prostatic epithelia require cytokine stimulation to produce MUC1. We will continue these studies by examining the effects of combined cytokine and DHT treatment on prostate cancer cell lines. These studies remain consistent with our original hypothesis that cytokines are important regulators of MUC1 expression in prostate cancer cells.

Task II – To define parameters by which interferon-γ or androgen modulate MUC1 association with β-catenin and GRB-2

These studies were originally planned to be initiated in months 16-22 and, therefore, have not been performed, yet.

Task III – To test effects of disruption of formation of MUC1 complexes with β-catenin and GRB-2 on prostate cancer cell growth in vitro

These studies were originally planned to be initiated in months 22-36 and, therefore, have not been performed, yet.

Key Research Accomplishments

1) Determination that neither interferon-γ, TNF-α nor DHT alone stimulates MUC1 expression in several prostate cancer cell lines
2) Determination that combined treatment of interferon-γ and TNF-α stimulates MUC1 expression in normal prostate epithelia
3) Determination that MUC1 and MUC1SEC mRNA are expressed by cytokine-treated normal prostate epithelia
4) Determination that cell-associated MUC1 in normal prostate epithelia is in the form of an SDS-dissociable complex of the transmembrane/cytoplasmic tail with the ectodomain

Reportable Outcomes

Extension of this work is the basis of a thesis for a Ph.D. candidate, John O'Connor, in the PI's lab.

Conclusions

It appears that neither normal prostate epithelia nor prostate cancer cell lines express high levels of MUC1 in their basal states. Normal prostate epithelia will do so when

stimulated with combinations of cytokines (interferon-γ and TNF-α) shown to greatly stimulate MUC1 expression in other cellular contexts. These observations suggest that synergistic actions of cytokines, and perhaps androgen, are required to augment MUC1 production in prostate cancer cells as originally proposed. This idea will be pursued in the next phase and will include examination of these responses at the level of the MUC1 mRNA, protein and promoter. Physiologically, these observations would be consistent with a model where MUC1 levels are high in primary tumors where paracrine factors maintain MUC1 expression. Furthermore, MUC1 is likely to be elevated in bone marrow metastases where cytokine levels are high. As discussed above, high level MUC1 expression is expected to provide protection from host immune surveillance. Understanding the molecular basis for this elevation in MUC1 expression should create novel avenues to interfere with these processes and retard cancer in bony metastases. Continued work examining the potential role of MUC1 as a mediator of signal transduction processes is expected to provide avenues to interfere with MUC1 function in MUC1-expressing cancer cells.

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Appendices

CV for Daniel D. Carson, Ph.D.

CURRICULUM VITAE

DANIEL D. CARSON, Ph.D.

Birth Date and Place: [redacted]

Citizenship: U.S.A

Social Security Number: [redacted]

Office Address: Department of Biological Sciences
University of Delaware
117 Wolf Hall
Newark, DE 19716

Telephone: (302) 831-6977
FAX: (302) 831-1033

Education:

B.S., 1975, University of Pennsylvania, Philadelphia, Pennsylvania: Biology

Ph.D., 1979, Temple University, Philadelphia, Pennsylvania: Microbiology

Postdoctoral Fellow; 1978-1983, The Johns Hopkins University School of Medicine, Baltimore, Maryland: Biochemistry

Academic and Professional Appointments:

9/98-Present, Professor and Chairman, Department of Biological Sciences, University of Delaware, Newark, Delaware 19716

9/93-9/98, Professor and Biochemist in Biochemistry and Molecular Biology Department, M.D. Anderson Cancer Center, Houston, Texas 77030

09/88-8/93, Associate Professor and Associate Biochemist, Department of Biochemistry and Molecular Biology, M.D. Anderson Cancer Center, Houston, Texas 77030

08/83-08/88, Assistant Professor and Assistant Biochemist in Biochemistry and Molecular Biology Department of Biochemistry and Molecular Biology, M.D. Anderson Cancer Center, Houston, Texas 77030

Editorial Boards:

Biology of Reproduction, 1997-2001; Frontiers in Bioscience, 1996-2000; Endocrinology, 1998-2002

Reviewer for:

Journal of Biological Chemistry, Developmental Biology, Journal of Histochemistry and Cytochemistry, Differentiation, Development, American Journal of Physiology, Journal of Cellular Physiology.

Committee Memberships:

Developmental Biology Study Section (Ad Hoc), NSF, 10/88; 10/97
Program Project Reviewer (Ad Hoc), NICHD, 04/90
Program Project Review, NIKDD, 10/90
Perinatal Emphasis Research Grant Review board, NICHHD, 5/91
Specialized Center for Research in Reproduction Site visit team, NICHD, 5/97; 9/97
Reproductive Biology Study Section, *ad hoc* member, 1992-1994
Reproductive Biology Study Section, member, 1995-1999
Texas Forum on Female Reproduction, Steering Committee, 1995-1998
Society for the Study of Reproduction Program Organizing Committee, 1997
Chairman, Serono Symposium on Embryo Implantation: Cellular, Molecular and Clinical Aspects, 1997
Scientific Advisory Board, Genosys Biotechnology, Inc., Woodlands, TX
Consultant, Advanced Tissue Sciences, San Diego, CA
Director, Texas Women's Reproductive Health Consortium, 1997

Honors and Awards:

Mayor's Scholarship, Philadelphia, PA, 1971-1978
Wolf Memorial Scholarship, 1971-1975
American Cancer Society Postdoctoral Fellowship, 1980-1981
Dean's Teaching Excellence List, GSBS, UTHSC, 1986-1995
Dean's Outstanding Teaching List. GSBS, UTHSC, 1996, 1997
H.E.B. Professorship in Cancer Research, 1997-1998
Trustees Distinguished Professor of Biology, 2000-present

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